CONCISE REPORT

Transforming growth factor β1 and insulin-like growth factor 1 block collagen degradation induced by oncostatin M in combination with tumour necrosis factor α from bovine cartilage

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Oncostatin M (OSM) in combination with tumour necrosis factor α (TNFα) promotes marked collagen breakdown from bovine cartilage in explant culture. This release was dependent upon matrix metalloproteinases and could be prevented by transforming growth factor β1 (TGFβ1) or insulin-like growth factor 1. Both growth factors reduced the expression and secretion of collagenase enzymes, and TGFβ1 induced tissue inhibitor of metalloproteinase production. This study shows for the first time that these anabolic growth factors can protect cartilage against OSM+TNFα induced destruction.

Progressive loss of cartilage matrix is a major characteristic of rheumatoid arthritis (RA). The proinflammatory cytokines interleukin 1 (IL1) and tumour necrosis factor α (TNFα) have been recognised as key pathogenic molecules in this process. One major effect mediated by these inflammatory cytokines in RA is the induction of matrix metalloproteinases (MMPs).[1] The MMPs are a family of zinc dependent endopeptidases that have the ability to break down all extracellular matrix components. Raised levels of several MMPs have been detected in sera and synovial fluids from patients with RA, and these levels correlate with disease activity and structural damage.[2 3] The activity of MMPs in tissues can be blocked by the tissue inhibitors of metalloproteinases (TIMPs). Blockade of MMP activity, therefore, represents a key control point in disease progression.

Oncostatin M (OSM) is a cytokine that has been found to induce joint inflammation and cartilage damage,[4 5] and has been localised to macrophages in rheumatoid synovium.[4] Moreover, raised levels of OSM are detected in RA synovial fluids,[4] and these levels correlate with joint inflammation and the markers of collagen and aggrecan degradation in RA.[6]

Transforming growth factor β1 (TGFβ1) and insulin-like growth factor 1 (IGF1) have important roles in cartilage matrix turnover because they both stimulate the synthesis of major matrix components,[7 8] and have been detected in articular cartilage and arthritis synovial fluids.[9 10] TGFβ1 blocks IL1 suppression of cartilage proteoglycan and collagen synthesis,[11] whereas IGF1 maintains the expression of type II collagen in the absence of serum without increasing type I collagen synthesis.[9] Recently, we showed that these growth factors can block cartilage breakdown induced by proinflammatory cytokines,[11–13] providing further evidence for a protective role in cartilage destruction.

We have shown that the combination of OSM+TNFα promotes a synergistic release of collagen from cartilage which is accompanied by a marked up regulation and activation of pro-collagenases.[13] This present study assesses the effects of TGFβ1 and IGF1 on OSM+TNFα induced cartilage degradation and MMP/TIMP expression in chondrocytes.

MATERIALS AND METHODS

Human recombinant OSM, TNFα, TGFβ1, and IGF1 were purchased from R&D Systems (Abingdon, UK). The metalloproteinase inhibitor BB-94 was provided by British BioTech Ltd (Oxford, UK). Human recombinant TIMP-1 protein and MMP/TIMP cDNAs were as previously described.[4 12]

Cartilage degradation assay

Because this study aims at assessing the protective nature of TGFβ1 and IGF1, we used an established assay of cartilage degradation, as previously described.[4] Although, there are differences between nasal and articular cartilages, we have previously shown that both types of cartilage respond to cytokines in a similar manner.[4] Culture supernates were harvested at day 7 and replenished with treatments identical to those on day 0, and the experiment continued until day 14. Day 7 and 14 supernates were stored at −20°C until assay. The remaining cartilage was papain digested, and stored at −20°C until assay. The doses of growth factors adopted in this study were as previously described.[11 12]

Collagen and collagenolytic activity assays

Culture supernates and cartilage digests were assayed for hydroxyproline as a measure of collagen, as previously described.[4] Collagen release was expressed as a percentage of the total. Collagenolytic activities (active and total) in culture supernates were determined by bioassay.[4] Statistical differences between sample group means were tested using the unpaired Student's *t* test.

Cell culture and northern blot analysis

Primary bovine nasal chondrocytes were extracted from cartilage as previously described.[12] Primary chondrocytes were cultured to 80% confluence, and serum starved overnight before stimulation with test reagents in serum-free medium as described. Total cellular RNA was assessed for the expression of MMPs and TIMPs essentially as previously described.[12]

Abbreviations: DMEM, Dulbecco's modification of Eagle's medium; IGF, insulin-like growth factor; IL, interleukin; MMPs, matrix metalloproteinases; OSM, oncostatin M; RA, rheumatoid arthritis; TGF, transforming growth factor; TIMPs, tissue inhibitors of metalloproteinases; TNFα, tumour necrosis factor α

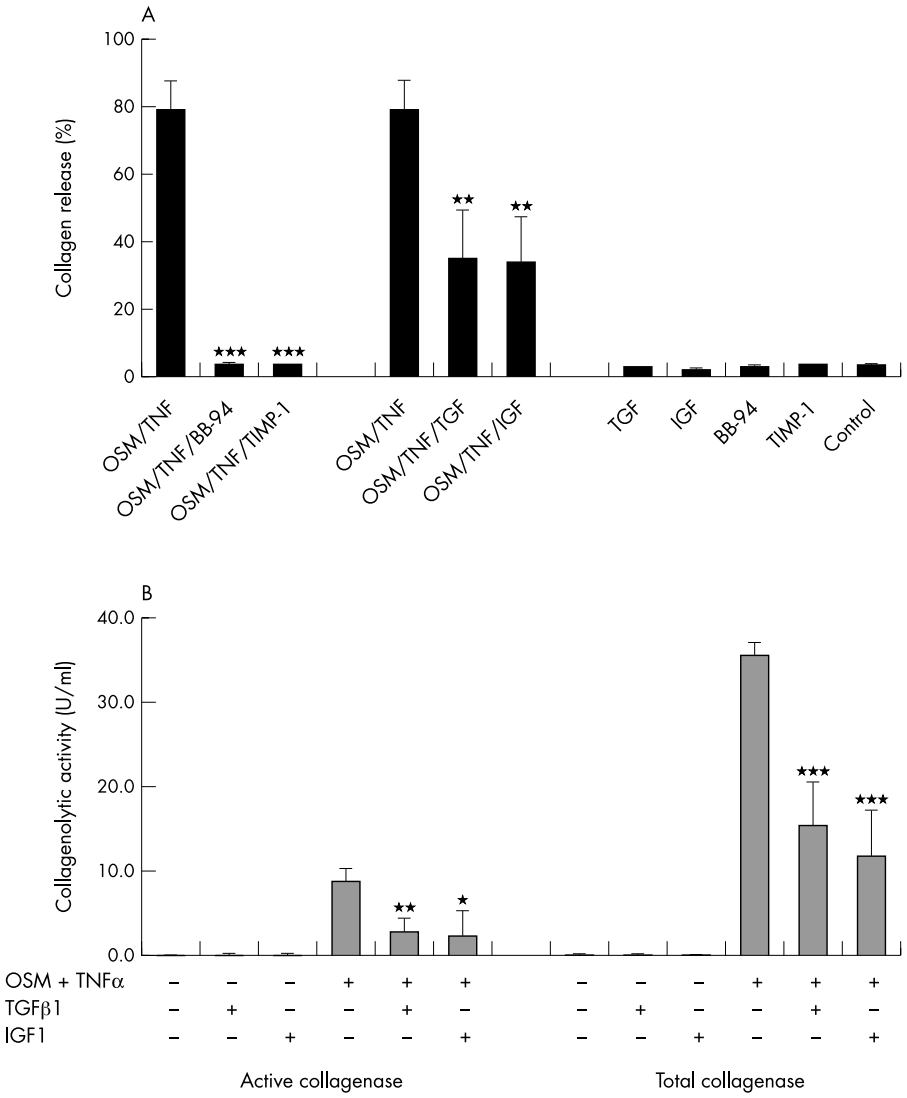

Figure 1 Blocking of OSM+TNFα induced collagen release and collagenolytic activity by growth factors and MMP inhibitors. Bovine nasal cartilage was stimulated with OSM+TNFα in the presence of TGFβ1 (10 ng/ml) or IGF1 (200 ng/ml) or BB-94 (10^{-5} M) or TIMP-1 (100 U/ml) for 14 days. The media were collected and replaced at day 7. The release of collagen into the media, and collagenolytic activity in the media were assayed as described in "Materials and methods". (A) The cumulative collagen release for the 14 day incubation is expressed as a percentage of the total collagen release for each treatment (mean (SD), n=4). (B) Levels of pro- and active collagenolytic activity (expressed as U/ml) are presented for each treatment. The experiments were done in duplicate. Significance was assessed by comparing the release of collagen or collagenolytic activity induced by OSM+TNFα with that for OSM+TNFα +TGFβ1 or IGF1, where *p<0.05, **p<0.01, ***p<0.001 using Student's *t* test.

RESULTS

Effects of growth factors and MMP inhibitors on collagen release and collagenolytic activity induced by OSM+TNFα from bovine nasal cartilage

We have previously shown that OSM alone does not induce collagen release,[4 11–13] whereas TNFα alone induces low (≤20%) collagen release.[13] However, OSM+TNFα (both at 5 ng/ml) reproducibly promoted significant collagen breakdown (≥75%) from bovine nasal cartilage; both TGFβ1 (10 ng/ml) and IGF1 (200 ng/ml) partially inhibited this release. This release was also completely inhibited by the synthetic metalloproteinase inhibitor BB-94 (10^{-5} M) as well as by TIMP-1 (100 U/ml), clearly indicating the involvement of MMPs in this process (fig 1A). High levels of collagenolytic activity were present in the culture medium of OSM+TNFα stimulated cartilage (fig 1B). TGFβ1 and IGF1 markedly reduced both the levels of total (pro- and active) collagenase activity, and most significantly the level of active collagenase activity (fig 1B).

Modulation of the expression of MMP and TIMP mRNA by TGFβ1 and IGF1 in OSM+TNFα treated bovine chondrocytes

Stimulation of bovine nasal chondrocytes with OSM+TNFα markedly induced the expression of MMP-1, MMP-3, and MMP-13. Furthermore, TIMP-1 expression was up regulated, TIMP-2 was slightly down regulated, and TIMP-3 expression was significantly down regulated (fig 2). Inclusion of either TGFβ1 or IGF1 significantly reduced the MMP expression. TGFβ1 alone, and when in combination with OSM+TNFα, up regulated TIMP-3 expression, but had no significant effects on the expression of TIMP-1 or TIMP-2. IGF1 had relatively little effect on OSM+TNFα induced TIMP expression other than to moderately decrease TIMP-1 (fig 2).

DISCUSSION

OSM+TNFα promoted significant release of collagen from cartilage, which was associated with an increase in collagenolytic activity as well as a decrease in TIMP expression.

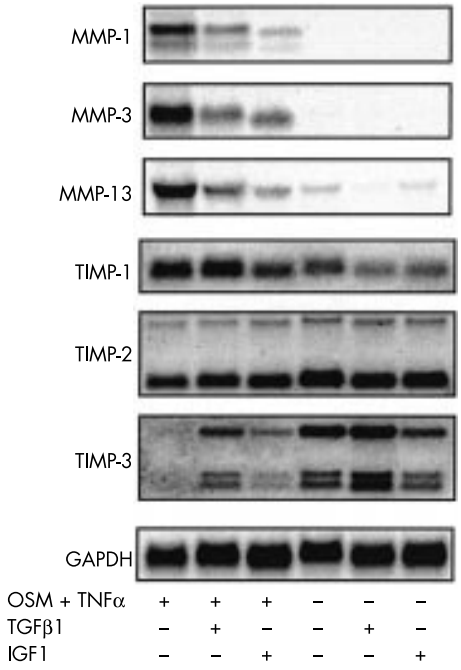

Figure 2 Modulation of MMP and TIMP mRNA expression by TGFβ1 and IGF1 in OSM+TNFα treated chondrocytes. Primary bovine nasal chondrocytes were cultured in Dulbecco's modification of Eagle's medium (DMEM) containing 10% fetal calf serum to 80% confluence. The medium was changed to serum-free DMEM overnight and then replaced with serum-free DMEM with OSM (5 ng/ml) +TNFα (5 ng/ml) with or without TGFβ1 (10 ng/ml) or IGF1 (200 ng/ml) for 24 hours. Total RNA was harvested and analysed by northern blot.

Moreover, OSM+TNFα induced the expression of the collagenolytic MMPs (MMP-1 and MMP-13) as well as an activator of pro-collagenases, MMP-3, in bovine chondrocytes. Because this collagen release was completely inhibited by the MMP inhibitors BB-94 and TIMP-1, these data strongly implicate increased MMP expression and reduced TIMP expression in OSM+TNFα mediated cartilage destruction.

TGFβ1 and IGF1 are both important anabolic growth factors in cartilage biology. Several studies have shown that both these growth factors exhibit protective and reparative properties in experimental models of joint destruction.[8 11–15] When TGFβ1 or IGF1 was combined with OSM+TNFα, collagen release from bovine nasal cartilage was significantly inhibited. Because this inhibition was associated with a marked reduction in both active and total collagenolytic activities, these data suggest that a decrease in the total amount of collagenase(s) produced had occurred, and that this was also associated with increased TIMP levels in the case of TGFβ1. Indeed, this may also account for the increased potency of TGFβ1 compared with IGF1, as we have shown previously.[11 12] Northern blot analyses confirmed this, as both growth factors reduced the mRNA levels of OSM+TNFα induced MMPs and TGFβ1 increased TIMP-3 expression. In conclusion, this study further highlights a potential role for these important growth factors in preventing cartilage damage in inflammatory joint diseases.

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